The transcriptional regulators Id2 and Id3 control formation of distinct CD8$^+$ memory T cell subsets

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Supplementary Figure 1. Generation of Id2-YFP knockin reporter. (a) i) The genomic structure of *Id2* with exons highlighted in green, the transcriptional start site indicated with an arrow, and the translational start and stop sites indicated by AUG and UAA respectively. Restriction enzyme sites utilized for southern hybridization screening are indicated, S = *ScaI*, H = *HindIII*, as well as the location of probes. ii) Homologous recombination of pBS-Id2 with H1-YFP-Neo-H2 in a bacterial system. Recombineering was employed to produce the targeting construct pBS-Id2-YFP-Neo. iii) Gene targeting of genomic Id2 with pBS-Id2-YFP-Neo in embryonic stem cells followed by deletion of the Neo cassette by *EIIa* driven *cre* recombinase in the mouse. **(b)** $Id2^{+/+}Id3^{+/+}$, $Id2^{Y/+}Id3^{+/+}$, and $Id2^{+/+}Id3^{G/+}$, $Id2^{Y/+}Id3^{G/+}$ mice were infected with VSV-OVA and antigen-specific $CD8^+$ T cells from peripheral blood lymphocytes (PBL) were analyzed at indicated days after infection. Bar graphs indicate percent of antigen specific $CD8^+$ T cells. Data are pooled from 5 independent experiments with n = 2 - 3 mice per group. Error bars indicate SEM.

Supplementary Figure 2. Expression of Id2-YFP and Id3-GFP in naive lymphocytes. (a) Thymocytes and splenocytes from naive $Id2^{Y/+}$ $Id3^{G/+}$ mice were analyzed for Id2-YFP and Id3-GFP expression. Representative flow cytometry plots from 4 independent experiments with 2 - 3 replicates. **(b)** Indicated populations were sorted from naive wild type C57BL/6 mice and expression of Id2 (left) and Id3 (right) transcripts were analyzed by qPCR. Data are representative of 2 independent experiments.

Supplementary Figure 3. Analysis of Id2-YFP and Id3-GFP reporter expression in endogenous $CD8^+$ T cells during infection. Flow cytometry plots of Id2-YFP and Id3-GFP

expression by polyclonal antigen specific T cells. *Id2*$^{Y/+}$*Id3*$^{G/+}$ mice were infected with **(a)** VSV-OVA or **(b)** Lm-OVA and on days indicated, antigen-specific CD44^{+} B220^{-} CD8^{+} T cells were identified by H-2K^{b}-OVAp tetramer staining of splenocytes. **(c)** Flow cytometry plots showing expression of KLRG1 and CD127 by Id3-GFP^{hi} and Id3-GFP^{lo} donor cells on day 7 or **(d)** 14 after infection with VSV-OVA or Lm-OVA as indicated. Numbers indicate percentage cells in each quadrant. Data are representative of 4 independent experiments with n = 1 - 3 mice per group.

Supplementary Figure 4. Correlation of CD25 and T-bet with Id2-YFP and Id3-GFP. (a) CD45.2^{+} C57BL/6 mice received *Id2*$^{Y/+}$*Id3*$^{G/+}$ CD45.1^{+} OT-I (2.5 x 10^{4}) cells 1 day before infection with Lm-OVA and CD8^{+} CD45.1^{+} splenocytes were analyzed at day 4 after infection. Bar graphs represent average MFI of Id2-YFP (left) and Id3-GFP (right) of CD25^{hi} and CD25^{lo} subsets. Data are representative of 4 independent experiments with n = 2-3 mice. **(b)** CD45.2^{+} C57BL/6 mice received *Id2*$^{Y/+}$*Id3*$^{G/+}$ CD45.1^{+} OT-I (5 x 10^{4}) cells 1 day before infection with VSV-OVA and CD8^{+} CD45.1^{+} splenocytes were analyzed at days 7 after infection. Representative histogram (left) and MFI (right) of T-bet expression by Id3^{lo} and Id3^{hi} cells. Unstained samples were used as negative control. Data are representative of 3 independent experiments with n = 2 - 3 mice. Error bars indicate SEM.

Supplementary Figure 5. Generation of *Id3*$^{G/G}$ OT-I mixed bone marrow chimeras. CD45.1^{+} Id3$^{+/+}$ or Id3$^{G/G}$ OT-I bone marrow were mixed equally with CD45.2^{+} C57BL/6 bone marrow and adoptively transferred into lethally irradiated C57BL/6 CD45.1.2^{+} hosts. CD8 and

CD44 expression by OT-I splenocytes from naive mice (left), chimeras after 12 weeks of reconstitution (middle), and purified for $CD44^{lo}$ populations before adoptive transfer (right).

Supplementary Figure 6. Expression of Id3-GFP and Id2-YFP by $CD8^+$ T cells after blocking with anti-IL-7Rα antibodies. Flow cytometry plots (left) and MFI (right) of Id2-YFP and Id3-GFP expression by $CD8^+$ OT-I T cells. $CD45.2^+$ C57BL/6 mice received $Id2^{Y/+}Id3^{G/+}$ $CD45.1^+$ OT-I (2.5×10^4) cells 1 day before infection with VSV-OVA and $CD8^+$ $CD45.1^+$ splenocytes were analyzed at day 5 after infection. Recipient mice were given 0.5 mg mAb A7R34 (anti-IL-7Rα) or control rat IgG intraperitoneally 1 day before infection and every other day after. Data are representative of 2 independent experiments with $n = 3$ mice per group. Error bars indicate SEM.

Supplementary Table 1. qPCR primers sequences

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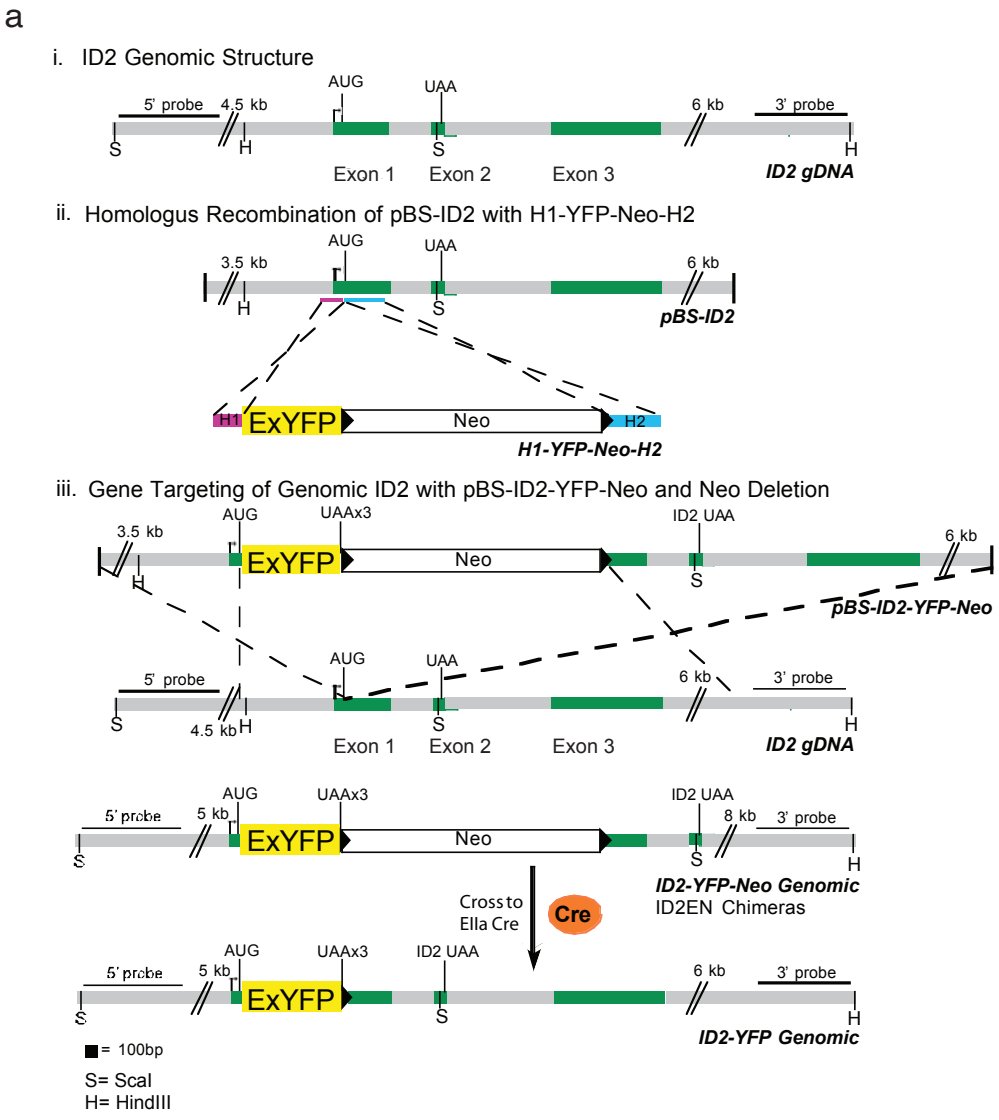
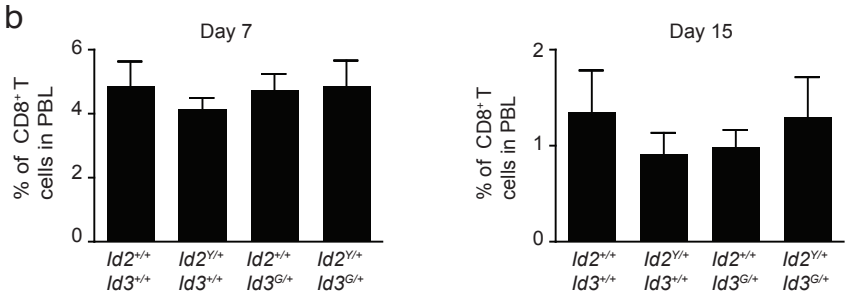
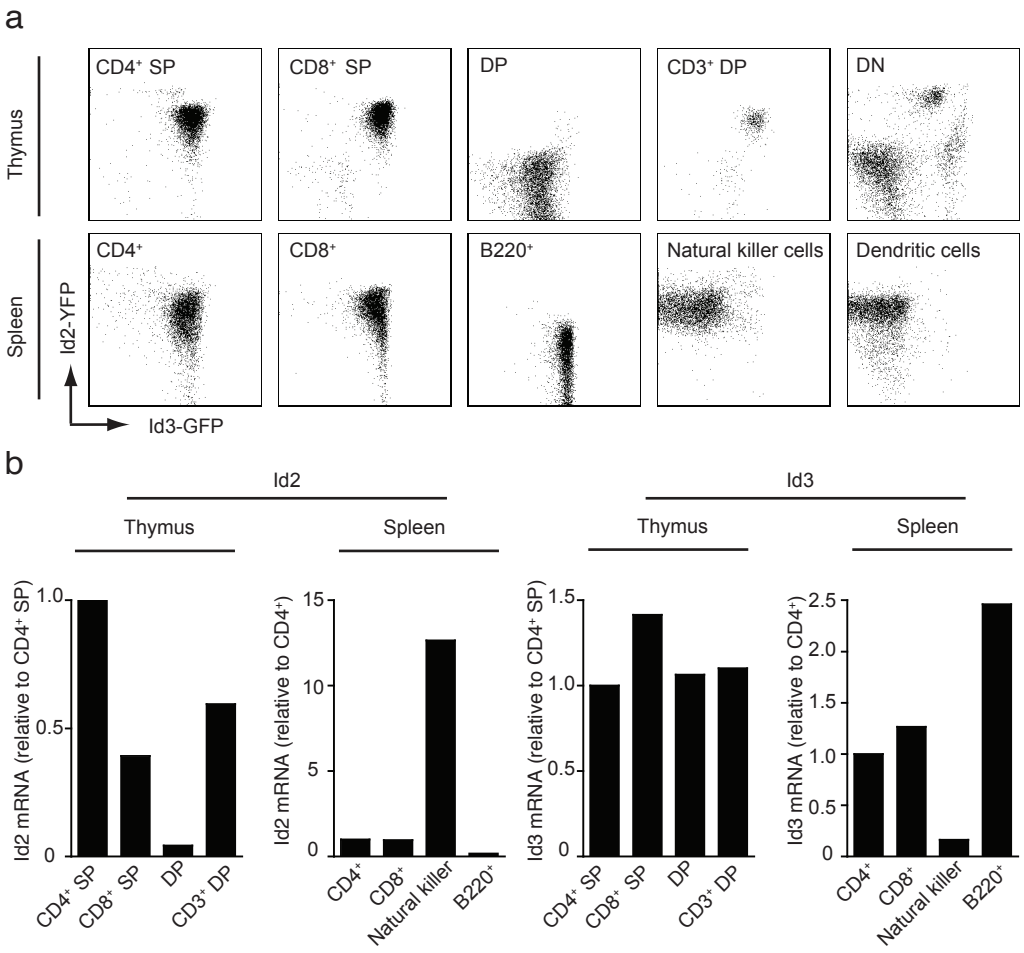
Supplementary Figure 1






a
Thymus
Spleen
CD4+ SP
CD8+ SP
DP
CD3+ DP
DN
CD4+
CD8+
B220+
Natural killer cells
Dendritic cells
Id2-YFP
Id3-GFP
b
Id2
Id3
Thymus
Spleen
Thymus
Spleen
Id2 mRNA (relative to CD4+ SP)
Id2 mRNA (relative to CD4+)
Id3 mRNA (relative to CD4+ SP)
Id3 mRNA (relative to CD4+)
CD4+ SP
CD8+ SP
DP
CD3+ DP
CD4+
CD8+
Natural killer
B220+

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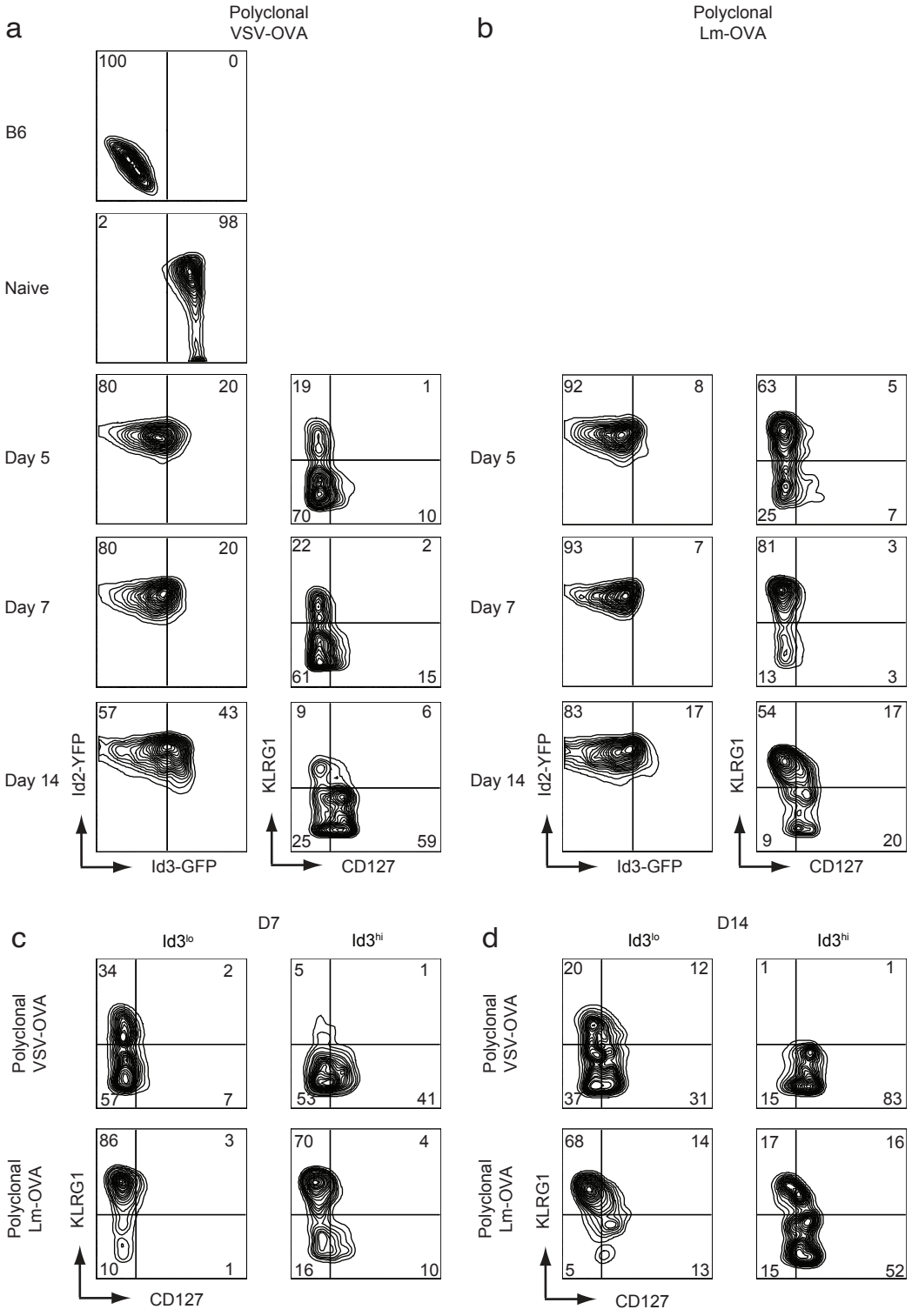
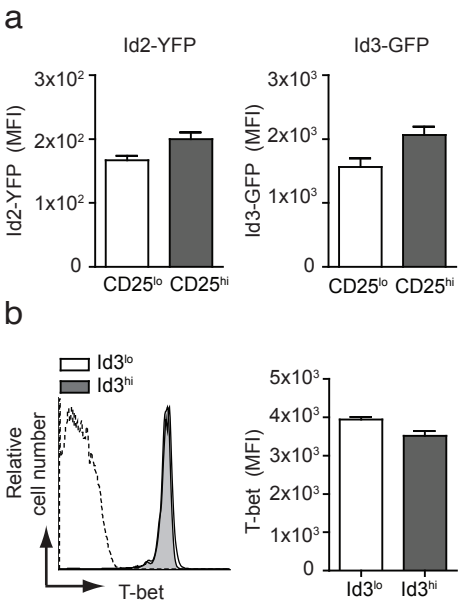
Supplementary Figure 3




a
Id2-YFP
Id3-GFP
3x10²
2x10²
1x10²
0
Id2-YFP (MFI)
CD25lo CD25hi
3x10³
2x10³
1x10³
0
Id3-GFP (MFI)
CD25lo CD25hi
b
Id3lo
Id3hi
Relative cell number
T-bet
5x10³
4x10³
3x10³
2x10³
1x10³
0
T-bet (MFI)
Id3lo Id3hi

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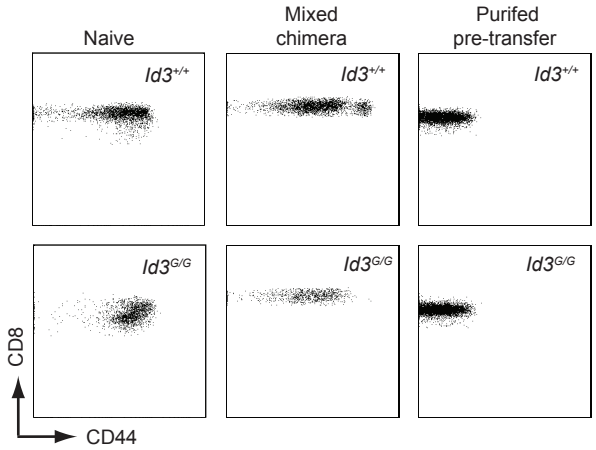
Supplementary Figure 5



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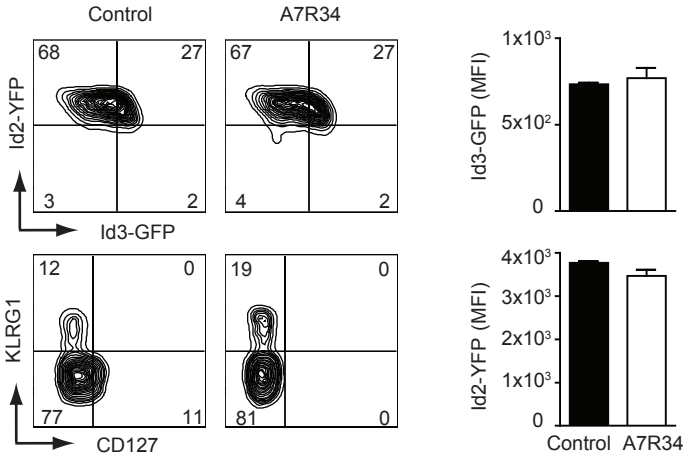
Supplementary Figure 6



Supplemental Table 1. qPCR primers

Name	Sequence
Bcl2 F	ACTTCGCAGAGATGTCCAGTC
Bcl2 R	TGGCAAAGCGTCCCCTC
Bcl6 F	CCTGTGAAATCTGTGGCACTCG
Bcl6 R	CGCAGTTGGCTTTTGTGACG
Bhlhe40 F	CTCAAACTTACTACTTTGGGTCAC
Bhlhe40 R	GAACATTTCTTGCCCTGCCT
Blimp1 F	ACATAGTGAACGACCACCCCTG
Blimp1 R	CTTACCACGCCAATAACCTCTTTG
Ccr7 F	ACAGCGGCCTCCAGAAGAACAGCGG
Ccr7 R	TGACGTCATAGGCAATGTTGAGCTG
CD62L F	CATTCCTGTAGCCGTCATGG
CD62L R	AGGAGGAGCTGTTGGTCATG
Cx3cr1 F	TCTTCATTGGCTTCTTTGGG
Cx3cr1 R	TCGTTGTCCTTTCTCTTTGTG
Cxcr5 F	TGGCCTTCTACAGTAACAGCA
Cxcr5 R	GCATGAATACCGCCTTAAAGGAC
Cxcr6 F	CTTCTCTTCTGATGCCATGGA
Cxcr6 R	GAAACACATCTGTCAGAGTCC
Fasl F	TGT CTC ATT GGC ACC ATC TT
Fasl R	GTG CCT CAA ACA TCC CTC TT
Gzmk F	TGGCTGGCGTTTATATGTCTTC
Gzmk R	TCTGGGAAACCAAGAGTAGCA
Id2 F	ACCAGAGACCTGGACAGAAC
Id2 R	AAGCTCAGAAGGGAATTCAG
Id3 F	GACTCTGGGACCCTCTCTC
Id3 R	ACCCAAGTTCAGTCCTTCTC
Ifng F	GAGCCAGATTATCTCTTTCTACC

Ifng R	GTTGTTGACCTCAAACTTGG
Il2 F	AGCAGGATGGAGAATTACAGGA
Il2 R	GAGGTCCAAGTTCATCTTCTAGG
Il2Ra F	CTCCCATGACAAATCGAGAAAGC
Il2Ra R	TCTCTTGGTGCATAGACTGTGT
Il7R F	AGTCCTCCTATGTGAGTCCT
Il7R R	ACCCATCTTCTTTGTGTTTCTG
Il12Rb2 F	GCTCTGCGAAATTCAGTACC
Il12Rb2 R	GGATCTGGAATGGTTCTGCT
Klrb1c F	GACACAGCAAGTATCTACCT
Klrb1c R	TACTAAGACTCGCACTAAGAC
Klrg1 F	ACCTCCAGCCATCAATGTTC
Klrg1 R	CCTCTGGACGAGGAATGGTA
Myb F	CATTTACAGCCCACTGGTACTC
Myb R	CCGTGACATTATCGCTTGTGTTC
Perforin F	CAC AAC ATC AAA GAA CAG GAG
Perforin R	CCT TAC TCT TCA GCT TTA GCA
St6gal1 F	GAACTCTCAGTTAGTCACCAC
St6gal1 R	AGAGATTTCCTGAATGATGTCC
Tbet F	AGCAAGGACGGCGAATGTT
Tbet R	GTGGACATATAAGCGGTTCCC
Zeb2 F	CAT GAA CCC ATT TAG TGC CA
Zeb2 R	AGC AAG TCT CCC TGA AAT CC
Hprt F	TGA AGA GCT ACT GTA ATG AT
Hprt R	AGC AAG CTT GCA ACC TTA ACC A